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Chronic biotoxin-associated illness: Multiple-system symptoms, a vision deficit, and effective treatment☆

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Abstract

Blooms of toxigenic organisms have increased in spatial and temporal extent due to human activities and natural forces that alter ecologic habitats and pollute the environment. In aquatic environments, harmful algal blooms pose a risk for human health, the viability of organisms, and the sustainability of ecosystems. The estuarine dinoflagellate, *Pfiesteria piscicida*, was discovered in the late 1980s at North Carolina State University as a contaminant in fish cultures. *P. piscicida* was associated with fish death in laboratory aquaria, and illness among laboratory workers who inhaled the mist above aquaria. Both the fish and humans exhibited signs of toxicity. During the 1990s, large-scale mortality among fish and other aquatic organisms was associated with high concentrations of *Pfiesteria* sp. in estuaries on the eastern seaboard of North America from New York to Texas. Illness among humans was associated with direct exposure to estuaries and exposures to estuarine aerosols around the time of *Pfiesteria*-related fish kills. This review of the scientific literature on associations between *Pfiesteria* and human illness identified some of the possible mechanisms of action by which putative *Pfiesteria* toxins may have caused morbidity. Particular attention was given to the *Pfiesteria*-associated, human-illness syndrome known as Possible Estuary Associated Syndrome (PEAS). PEAS was characterized by multiple-system symptoms, deficits in neuropsychological tests of cognitive function, and rapid and severe decrements in visual contrast sensitivity (VCS), an indicator of neurologic function in the visual system. PEAS was diagnosed in acute and chronic illness cases, and was reacquired during re-exposure. Rapid normalization of PEAS signs and symptoms was achieved through the use of cholestyramine therapy. Cholestyramine, a non-absorbable polymer, has been used by humans to lower cholesterol levels since it was approved for that use by the U.S. Food and Drug Administration in 1958. When dissolved in water or juice and taken orally, cholestyramine binds with cholesterol, bile acids, and salts in the intestines, causing them to be eliminated rather than reabsorbed with bile during enterohepatic recirculation. Cholestyramine also has been reported to bind and eliminate a variety of toxic substances. The efficacy of cholestyramine therapy in treatment of PEAS supported the hypothesis that PEAS is a biotoxin-associated illness.

Keywords: *Pfiesteria*; Biotoxins; Toxigenic microorganisms; Possible Estuary Associated Syndrome; Visual contrast sensitivity

1. Background

Human activities and natural forces that pollute the environment and alter ecologic habitats can trigger a rapid expansion in the population size of toxigenic microorganisms in marine water, estuaries, fresh water and on land (Fig. 1). Toxigenic microorganisms produce biotoxins, secondary metabolites that are not essential for viability of the microorganisms, but often provide an important advantage during competition among organisms for predominance in an ecologic niche. Blooms of toxigenic microorganisms

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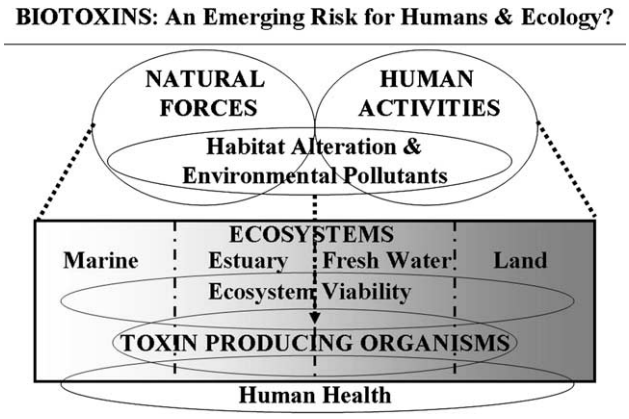

Fig. 1. Biotoxins, toxins made by living organisms, may pose an emerging risk to human health and ecology. Both human activities and natural forces cause pollution of the environment and alterations in habitats that can support toxigenic organisms. Evidence indicates that the spatial and temporal extent of populations of toxigenic organisms is increasing in marine, estuarine, and fresh waters, as well as on land. Biotoxins produced by these organisms adversely impact human health and the viability of ecosystems. Recent blooms of the estuarine dinoflagellate, *Pfiesteria* sp., have been associated with massive fish kills and human illness along the Eastern Seaboard of the United States.

have increased in spatial and temporal extent in recent years, particularly in aquatic environments [89]. Toxigenic blooms, therefore, pose an emerging risk for human health, the viability of organisms, and the sustainability of ecosystems. Recent associations of species in the dinoflagellate genera, *Pfiesteria*, with large scale mortality among estuarine organisms and morbidity among humans indicate that *Pfiesteria* sp. is one example of an emerging risk from toxigenic blooms.

Pfiesteria piscicida was first discovered as a contaminant in fish cultures at North Carolina State University. The fish were cultured in aquaria containing water collected from North Carolina estuaries. *P. piscicida* was identified following demise of the fish due to unknown causes [58]. A second member of the toxic *Pfiesteria* complex (TPC), *Pfiesteria shumwayae*, was identified in 2001 [30]. More than 2000 fish-bioassay studies were conducted with clonal TPC and axenic (i.e., germ-free) algal prey in two laboratories [17,52]. The data indicated that TPC had a complex, multi-staged life cycle and distinct functional sub-types, only some of which killed fish. TPC blooms were stimulated by inorganic nutrient enrichment [14,16,28], and transition to a toxic stage was associated with the presence of fish tissues or secretions [13,52]. Field investigations subsequently associated *P. piscicida* with more than 50 major fish kills in estuaries on the eastern coast of the United States [14,15,29,59]. The fish appeared narcotized, displaying lethargic behavior, a poor fright response, lesions, hemorrhage, and ultimately death. Water samples indicated that *P. piscicida* was present at concentrations ranging from 600–35,000 cells/ml in waters ranging in temperature from 9–31° C and in salinity from 0–30 psu during fish kills. Much lower concentrations of *P. piscicida* were found only hours after fish kills due to *P. piscicida*'s encystment and settlement into sediment [14]. Molecular probes were developed to detect TPC and assess its geographic range, which extended from New York to Texas [7,60,68]. Both toxic *P. piscicida* and *P. shumwayae* were subsequently isolated from sediment collected from northern European waters, demonstrating a wide geographic distribution [42]. To date, however, fish kills have not been attributed to *Pfiesteria sp.* in Europe [42].

The first indication that exposure to TPC posed a risk to human health came from illness among investigators working with *Pfiesteria sp.* in the laboratory [28]. The exposure of laboratory personnel to aerosols from ichthyotoxic (i.e., fish killing) TPC cultures was associated with chronic, multiple-system illness involving impaired function of the central and autonomic nervous systems, pulmonary distress, hepatic and renal dysfunction, and immunologic suppression [28]. The most severely affected individual subsequently developed blindness in one eye that was attributed to TPC exposure [70]. An in vivo rodent model of cognitive effects from exposure to TPC cells and filtrates [46–50,65] implicated hippocampal dysfunction as the cause of learning impairment [50]. In vitro receptor binding studies indicated that TPC filtrates inhibit NMDA-receptor binding in mammalian brain [25].

Other in vitro studies using rat GH4C1 pituitary cells that expressed voltage-dependent calcium, but not sodium, channels, an Adenosine-5′-triphosphate (ATP) P2X7-like receptor, and c-*fos*-luciferase genes indicated that a putative *Pfiesteria* toxin [pPfTx; [55]] mimicked the kinetics of cell permeabilization caused by ATP's action on P2X7 receptors [26,27,45,53]. pPfTx acting on purinergic P2X7 receptors inducted the formation of a nonselective cation channel, causing elevation of cystolic free calcium and permeabilization of the cell to progressively larger ions, resulting in cell lysis [53]. These mammalian in vivo and in vitro studies provided insight into some of the mechanisms of action that may be responsible for the adverse health effects attributed to the exposure of humans to environments inhabited by *Pfiesteria*.

The remainder of this article reviews the scientific literature on associations between human contact with *Pfiesteria*-inhabited estuaries and human morbidity, with a focus on the diagnosis of Possible Estuary Associated Syndrome [PEAS; [20]], thought to be a biotoxin-associated illness. Two particularly important points are indicated by the evidence presented. First, the onset of PEAS is accompanied by a large and rapid loss of visual contrast sensitivity (VCS). VCS, an indicator of neurologic function in the visual system, is a measure of the least amount of luminance contrast between darker and lighter areas of a pattern that is necessary for a viewer to distinguish the pattern from a homogeneous field. Second, PEAS can be resolved within days by therapeutic administration of cholestyramine, a non-absorbable polymer with anion-

binding capacity, to eliminate toxins. VCS returns to normal during recovery as symptoms resolve.

2. Human cases from environmental exposure

A series of fish kills was associated with *Pfiesteria* through measurement of high cell concentrations in the estuaries of the Chesapeake Bay, Maryland, during the summer of 1997. Reports of adverse health effects among people in physical contact with the estuaries [72,73] led to the initial study that described a human illness syndrome associated with *Pfiesteria* [31]. A single-blind, case-control, clinical investigation was undertaken by a group of Maryland physicians and researchers [31]. The exposed study participants received thorough medical and laboratory assessments, and completed questionnaires on symptoms, medical history, and exposure to toxic substances. Estimates of the amount of time spent in contact with the estuaries during fish-kill periods were derived from questionnaire data. Neuropsychological tests were administered to the exposed study participants and to age- and occupation-matched controls. The most common signs and symptoms among cases were memory loss, confusion, decreased assimilation of new information, headache, skin rash, burning skin upon estuary–water contact, eye and conjunctival irritation, sensitivity to bright light, abdominal pain, secretory diarrhea, and bronchospasm. The neuropsychological data showed statistically-significant differences between case and control cohorts in verbal learning and memory, resistance to interference and selective attention, motor speed, and dexterity. Statistical analyses showed significant linear trends between worse test performance and increased time spent in the TPC-inhabited estuaries. Although the degree of recovery among cases could not be determined because premorbid data were unavailable, most test scores were within normal limits 3–6 months following exposure. The study results indicated that humans in contact with *Pfiesteria*-inhabited estuaries were at risk for a clinical syndrome characterized by multiple-system symptoms and deficits in cognitive function. The severity of the syndrome was positively associated with the degree of exposure.

Illness was also reported by seven employees of the state of Maryland who investigated a *Pfiesteria*-related fish kill in the estuaries [32]. The workers reported flu-like symptoms following little or no direct contact with the water, and only about 4 h of aerosol exposure. Results from the small case-control study showed statistically-significant group differences in headache, sensory irritation, sore throat, abdominal pain, nausea, and diarrhea. These studies [31,32] helped the Centers for Disease Control and Prevention define PEAS as a multiple-system illness [19,20]. The case definition was: 1) symptoms criteria—memory loss or confusion of any duration, and/or three or more selected symptoms (headache, skin rash at the site of water contact, sensation of burning skin, eye irritation, upper respiratory irritation, muscle cramps, gastrointestinal symptoms) that, with the exception of skin rash and burning skin sensation, persisted for >2 weeks; 2) exposure potential—symptoms reported within 2 weeks of exposure to estuarine waters; and 3) absence of confounders—a health care provider could not identify another cause of the symptoms.

Shortly thereafter in the fall of 1997, the state of North Carolina undertook a cross-sectional, clinical study of watermen who did (estuary workers) and did not (offshore workers) have potential to contact TPC-inhabited estuaries. Because most of the potentially exposed watermen had not contacted *Pfiesteria*-related fish kills for more than a year, the goal of the study was to determine if evidence for chronic effects from past exposure could be discovered [82]. Eight of 22 estuary workers were identified as "recommended for follow-up" due to possible *Pfiesteria*-related effects, whereas only three of 21 offshore workers received the same classification. Mild peripheral neuropathy was diagnosed in 37% of the estuary workers, but in only 19% of the offshore workers. However, no statistically significant group differences were observed on neuropsychologic or computerized tests of neurobehavioral function. As shown in Fig. 2, visual contrast sensitivity (VCS), an indicator of neurologic function in the visual system, showed a large deficit in the estuary cohort relative to the offshore cohort, a statistically significant difference [38,39]. The cohorts did not differ in visual acuity, an indication of comparable optical refraction in the two groups. Several analyses were undertaken to investigate the possibility that factors other than work location might account for the group difference in VCS. First, neuropsychologists determined that approxi-

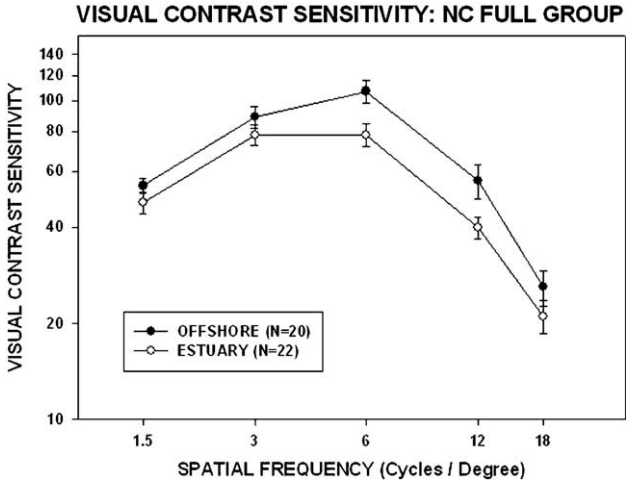

Fig. 2. The full occupational group in the North Carolina study [33,39]. Visual contrast sensitivity (mean+SEM) functions for the estuary (exposed to *Pfiesteria*-inhabited estuaries) and offshore (unexposed) cohorts. MANOVA analyses indicated that the group factor and the group-by-spatial frequency interaction term were significantly different. Step-down tests indicated that the VCS scores of the estuary cohort at 6 and 12 cycles per degree of visual arc were significantly lower than that of the offshore cohort.

mately half of the study participants in both cohorts had a medical or lifestyle factor that might influence neurologic function. The VCS data were re-analyzed after excluding those participants. The group difference in VCS, however, remained statistically significant and increased in magnitude (Fig. 3). Second, multiple linear-regression analyses indicated that the group difference in VCS was not accounted for by differences in age, education, smoking, alcohol consumption, total time spent on any body of water (a surrogate indicator of bright sunlight exposure), and their first order interaction terms. Third, an assessment of questionnaire data indicated that the cohorts did not differ in the frequency of occupational exposure to mercury, lead, other metals, pesticides, fumes, or solvents, or in total years of solvent exposure. An exploratory analysis indicated a statistically-significant linear relationship between hours spent at estuarine fish kills and decreased VCS. This dose–response relationship and the VCS difference between cohorts with and without exposure to *Pfiesteria*-inhabited estuaries were subsequently verified in a study conducted in Virginia estuaries [41,83]. These results led the "Peer Review Panel on the state of the science concerning *Pfiesteria*", established by the CDC, to conclude that VCS can be used to indicate neurologic alterations in patients exposed to *Pfiesteria* [69]. The need for cautious interpretation was noted by others who attended the CDC National Conference on *Pfiesteria*, because VCS deficits are not "toxin-specific" [67]. For example, VCS deficits have been associated with occupational exposure to a mixture of airborne solvents, and with environmental exposure to the dry cleaning solvent, tetrachloroethylene (i.e., perchloroethylene or perc) [9,37,38,71].

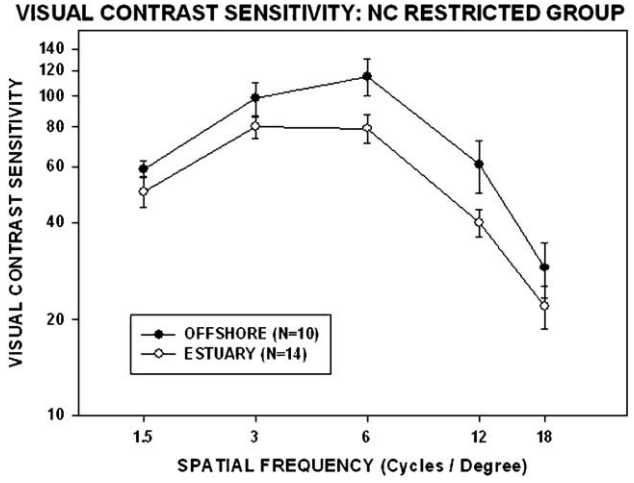

Fig. 3. The restricted occupational group in the North Carolina study [33,39]. Visual contrast sensitivity (mean+SEM) functions for the estuary (exposed to *Pfiesteria*-inhabited estuaries) and offshore (unexposed) cohorts in the North Carolina study restricted to include only participants free of potentially confounding factors. MANOVA analyses indicated that the group factor and the group-by-spatial frequency interaction term were significantly different. Step-down tests indicated that the VCS score of the estuary cohort at 6 cycles per degree of visual arc was significantly lower than that of the offshore cohort.

After the state of North Carolina and the U.S. Environmental Protection Agency issue a joint press release announcing the VCS results from the North Carolina study, VCS testing was implemented in two PEAS investigations [74,76], and subsequently in the CDC sponsored state surveillance studies in North Carolina, Virginia, and Maryland [54]. Shoemaker and Hudnell [76] reported a series of cases that met the CDC criteria for PEAS, and demonstrated a spectrum of multiple-system symptoms and large VCS deficits during acute, chronic and repeated-acquisition illnesses. The VCS deficits resolved as symptoms abated within 2 weeks of therapy with cholestyramine [76]. Cholestyramine is a nonabsorbable polymer that binds and eliminates cholesterol and a variety of toxins from bile in the intestines [1,8,10,11,18,22,23,40,51,56,57,62,64,84,87], preventing toxin reabsorption with bile during enterohepatic circulation [3,44]. The efficacy of cholestyramine therapy supported the hypothesis that PEAS was a biotoxin-associated illness.

Following the case-series report [76], a study was conducted to address the hypothesis that residential and recreational contact with *Pfiesteria*-inhabited estuaries can cause PEAS [74]. Three cohorts were assembled from patients who sought medical attention at Shoemaker's clinic in Pocomoke City, Maryland. The estuary cohort ($N=77$) had potential for exposure because of residence on the shores of *Pfiesteria*-inhabited estuaries or due to frequent recreational activity on those estuaries. The marine cohort ($N=34$) resided by, or recreated on, the ocean but not estuaries, and did not have exposure potential. The land cohort ($N=53$) had no exposure potential due to the lack of contact with any body of water. Prior to inclusion in the study, all study participants were screened for exclusion using physician-administered questionnaires. Potential participants were excluded from study participation due to potentially confounding factors, including serious ongoing illness or neurologic disease, alcoholism, occupational exposure to solvents, petroleum products, metal fumes, or pesticides, previous diagnoses of a PEAS-like illness, Lyme disease, *Ciguatera* seafood poisoning, chronic soft-tissue injury, exposure to mold in the indoor environment, and other medical, environmental, and lifestyle factors. All participants were assessed using a physician-administered symptom questionnaire, and visual acuity and VCS tests. The two non-exposed, control cohorts did not differ in group-mean number of symptoms, years of education, visual acuity or VCS, and were combined into one control cohort ($N=87$) for comparison with the estuary cohort. All members of the estuary cohort underwent a medical examination, pulmonary function testing, and blood analyses. Blood analyses included a complete blood count and a comprehensive metabolic profile. Differential diagnosis techniques were used to determine whether or not a cause of illness other than PEAS could be identified. Although the physician was aware of each participant's cohort assignment, the use of objective indicators helped to minimize the

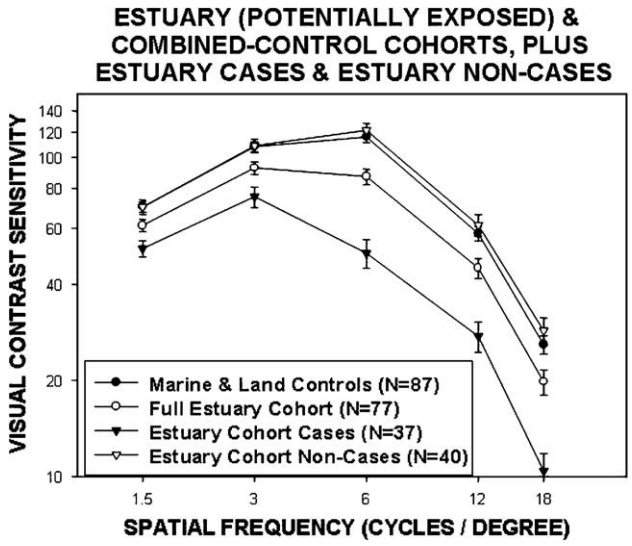

Fig. 4. Residential and recreational exposure in Chesapeake Bay estuaries [74]. VCS spatial frequency profiles for the combined-control cohort, the full estuary cohort, and the estuary cohort divided into PEAS cases and non-cases. Statistical analyses indicated that the estuary and combined-control cohorts were not significantly different in visual acuity, but that the two cohorts differed significantly in overall mean contrast sensitivity, mean contrast sensitivity at 6 and 12 cycles/degree, and the shape of the contrast sensitivity profiles. These effects were attributable to VCS deficits in the PEAS cases identified in the estuary cohort. VCS in estuary cohort non-cases was comparable to that of combined-control cohort.

potential for physician bias to influence the diagnostic classification.

The estuary and combined-control cohorts were not statistically different in age, gender, years of education, or visual acuity [74]. VCS, however, was reduced by about 33% in the estuary cohort relative to the combined-control cohort, a statistically significant difference (Fig. 4). In the estuary cohort, 37 of the 77 members met the CDC criteria for designation as a PEAS case. VCS in the PEAS cases was reduced by about 60% relative to the combined-control cohort, whereas VCS in the estuary non-cases was indistinguishable from that of the combined-control group (Fig. 4). The peak of the contrast sensitivity function was shifted from mid-spatial frequency (6 cpd) to the next lower spatial frequency (3 cpd) in the PEAS cases (Fig. 4). Symptom prevalence in the PEAS cases prior to therapy was much higher than in all non-cases, a combination of the estuary non-cases and the combined-control cohort (Table 1). A letter of exemption for the use of cholestyramine in the study was granted by the U.S. Food and Drug Administration (FDA). Cholestyramine was prescribed for the PEAS cases, who self-administered 9 g of cholestyramine, dissolved in apple juice or water, 4 times a day for 2 weeks. Cholestyramine was taken at least 1 h after, and ½ h prior to, eating or taking other medications. Sorbitol and Prilosec were taken as needed by a few of the PEAS cases to control the medication side effects of constipation and acid reflux, respectively. Other occasional side effects of therapy were abdominal distension and flatulence. Evaluation at re-examination indicated good therapeutic compliance.

All PEAS cases noted marked health improvement at re-examination after 2 weeks of therapy [74]. VCS was at the level of the combined-control cohort, a statistically significant improvement (Fig. 5). In addition, the shape of the contrast sensitivity function normalized, showing a statistically-significant shift in peak sensitivity to mid-spatial frequency (Fig. 5). Symptom prevalence was also dramatically improved; the prevalence of all symptoms was decreased, and prevalence was at or near the level seen in all non-cases for most symptoms (Table 1). The results of this study supported the hypothesis that residential or recreational contact with *Pfiesteria*-inhabited estuaries is a risk factor for the development of PEAS. Although the PEAS cases were aware that they were taking medication, results from the double-blind, placebo-controlled, crossover, clinical trial described below indicated that improvement in the PEAS cases was not likely due to a placebo effect or natural recovery over time.

Two control studies were conducted on the use of cholestyramine therapy [74]. The first control study assessed the effect of cholestyramine therapy on VCS in two populations, healthy individuals ($N=15$) and hypercholesterolemia patients ($N=8$). Two weeks of cholestyr-

Table 1
Symptoms in the Chesapeake Bay Estuaries Study on PEAS

	CDC																	
	Memory	Confusion	Headache	Skin rash	Burning skin	Eye irritation	Upper respiratory	Muscle cramp	Gastrointestinal	Concentration	Light sensitive	Cough	Short of breath	Fatigue	Muscle pain	Weakness	Abdominal pain	Vertigo
Cases (N=37)																		
% Before Rx	84	24	73	16	8	68	41	14	57	35	68	43	57	70	43	35	41	16
% After Rx	14	3	8	0	0	0	5	3	5	0	24	5	5	5	14	0	0	3
Non–Cases (N=127)																		
% Before Rx	2	0	4	0	0	0	3	0	1	0	2	5	2	2	3	0	2	2
% After Rx	2	0	2	0	0	0	2	0	0	0	1	3	2	2	1	0	2	1

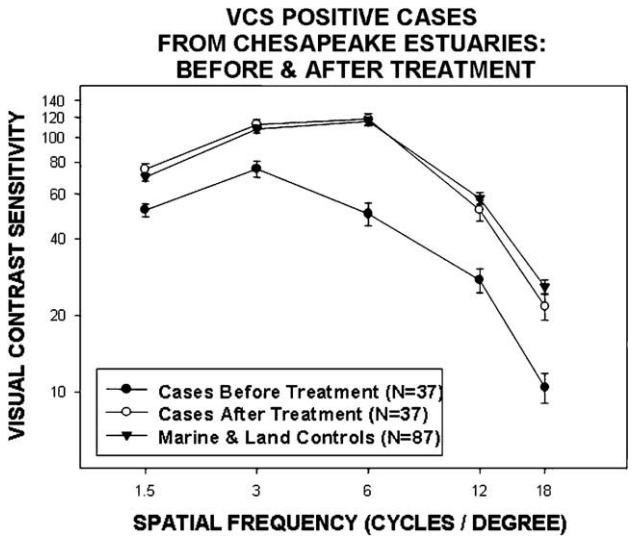

Fig. 5. Treatment of PEAS cases in the Chesapeake Bay study [74]. VCS spatial frequency profiles for the PEAS cases identified in the estuary cohort before and after treatment with cholestyramine and the combined-control cohort. Statistical analyses indicated that treatment did not significantly alter visual acuity, but that treatment significantly increased overall mean contrast sensitivity, and mean contrast sensitivity at each spatial frequency. A shift in peak contrast sensitivity to mid-spatial frequency restored the shape of the contrast sensitivity profile to normal.

amine therapy with the same dosage schedule used by the PEAS cases did not have a statistically significant effect on VCS or visual acuity in either group (Fig. 6). These results indicate that cholestyramine therapy does not improve VCS in people that have not been exposed to biotoxins, and that VCS does not improve simply due to retesting. The second control study assessed the effects of cholestyramine on PEAS cases ($N=8$) in a double-blind, placebo-controlled, crossover, clinical trial. VCS prior to cholestyramine therapy was reduced by about 70% at mid-spatial frequency, and the peak of the contrast sensitivity function was shifted from mid-spatial frequency to the next lower spatial frequency (Fig. 7). The group that took the placebo for 2 weeks prior to cholestyramine therapy showed no improvement in visual acuity or VCS following placebo treatment. However, VCS normalized without acuity changes after 2 weeks of cholestyramine therapy (Fig. 7). Symptom prevalence was not reduced following placebo treatment, but was reduced by 90% following cholestyramine therapy. The group that took cholestyramine for 2 weeks prior to taking the placebo also showed VCS normalization after cholestyramine therapy. That group maintained a normal VCS level without further improvement after being treated with the placebo for 2 weeks. Symptom prevalence decreased by 83% following cholestyramine therapy, and did not decrease further following 2 weeks of placebo treatment. The results of these studies indicated that cholestyramine therapy did not affect VCS in healthy individuals or in hypercholesterolemia patients, and that PEAS cases responded to cholestyramine therapy, but not to placebo treatment. This evidence supported the hypothesis that PEAS is a biotoxin-associated illness because cholestyramine has no known therapeutic benefit other than the elimination of cholesterol and toxins. However, because

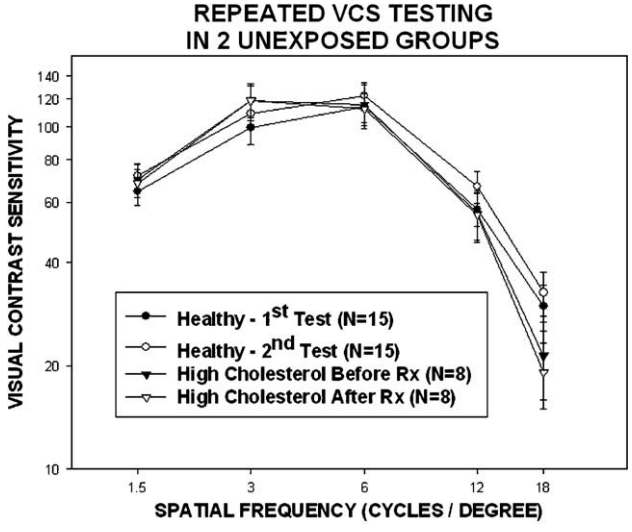

Fig. 6. Spatial frequency profiles for healthy and hypercholesterolemia cohorts tested at the beginning and end of a 2 week interval of cholestyramine therapy [74]. Statistical analyses indicated that contrast sensitivity did not change significantly during the interval in either group. These results indicated that therapy does not affect VCS in unexposed cohorts, and that repeated testing alone does not cause contrast sensitivity to increase. Contrast sensitivity was similar in the two groups except that sensitivity was slightly lower at 18 cycles/degree in the high cholesterol group than in the healthy group, likely reflective of an age-related reduction in visual acuity in the high cholesterol group.

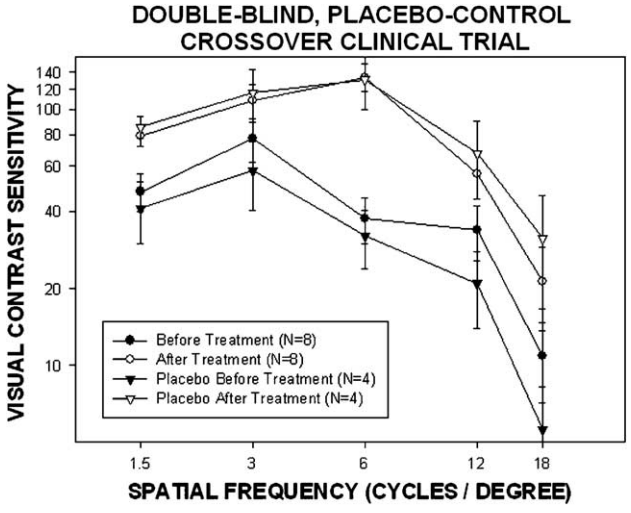

Fig. 7. Group-mean spatial frequency profiles of visual contrast sensitivity from the double blind, placebo controlled crossover clinical trial [74]. VCS before treatment was strongly depressed relative to after cholestyramine treatment in whole group. The group that took a placebo for 2 weeks prior to cholestyramine treatment showed no improvement after placebo, but marked improvement after cholestyramine treatment. The group that took cholestyramine first showed a marked improvement in VCS that was retained after the placebo condition of the trial was completed.

tests for the putative *Pfiesteria* toxin, pPfTx, were unavailable, exposure to the toxin could not be measured. This study limitation precluded the conclusion that PEAS was caused by exposure to *Pfiesteria*. It remained possible that exposure to *Pfiesteria*-inhabited estuaries was associated with PEAS due to some unknown factor.

3. Discussion

The studies discussed above indicate that contact with *Pfiesteria*-inhabited estuaries around the time of fish kills was a human health risk. PEAS was characterized by multiple-system symptoms and a large VCS deficit greatest at the mid-spatial frequency and the next-highest-spatial frequency. PEAS cases showed a robust and rapid response to cholestyramine therapy. The incidence and prevalence of PEAS was unknown. The residential and recreational study discussed above [74] did not use population-based sampling techniques or distinguish acute from chronic PEAS and was, therefore, unable to address these issues. Although PEAS was associated with *Pfiesteria*-inhabited estuaries and fish kills [31], the cause of PEAS remained unknown. A toxin produced by *Pfiesteria* was isolated, but the crystal structure was not identified [45,53,55]. The issue of toxin production by *Pfiesteria* continues to be a topic of intense controversy [12,43]. Attribution of PEAS to a particular organism and toxin or toxins must await the isolation of the toxin(s) from an estuarine organism, the identification of that toxin(s) in humans with PEAS, and the demonstration of its absence in recovered PEAS cases and other healthy individuals.

The VCS test provides a quick, noninvasive, and inexpensive tool for assisting in the diagnosis of PEAS and recovery monitoring during cholestyramine therapy. VCS deficits, like deficits on neuropsychological tests, are nonspecific in that there are many possible causes of those deficits. Therefore, a thorough investigation of other possible causes of deficits in these tests must be undertaken by a physician before a diagnosis of PEAS can be made. It is particularly important to note that chronic, organic-solvent exposure is associated with VCS deficits [9,37,38,71], as well as with cognitive deficits, that are not reversed by cholestyramine or other therapies [35,36]. It is also important to note that VCS deficits can cause deficits in neuropsychological tests that rely on small, briefly presented visual stimuli and measurement of short response times [38]. A better understanding of injury in neurologic pathways can result from assessments based on both VCS and neuropsychological test scores. Because VCS deficits are nonspecific, and because the cause of PEAS is unknown, the use of VCS testing in assessment of PEAS cases has raised controversy [34,35,79–81,]. However, with proper screening for other causes of deficits in VCS and neuropsychological tests, these tools provide valuable, objective indications of neurologic impairment in a condition otherwise characterized only by non-specific symptoms.

The VCS test does have advantages over neuropsychological tests, however, in that VCS assessments can be completed within a few minutes, and medical personnel can be trained to administer the VCS test according to a standardized protocol in a relatively short period of time. Furthermore, the visual system has only a few functional outputs, photopic and color vision that are cone-mediated, scotopic vision that is primarily rod-mediated, central vision, peripheral vision, motion detection, and the detection of patterns defined by luminance contrast. Each of these functional outputs can be readily quantified using relatively simple tests, such as use of the VCS test to measure visual-pattern contrast thresholds (visual contrast sensitivity is the inverse of visual contrast threshold, $1/(L_{Max}-L_{Min}/L_{Max}+L_{Min})\times 100$, where L_{Max} and L_{Min} are the luminances of the darker and lighter areas of the pattern, respectively). Conversely, the cognitive system has a very large, unknown number of functional outputs that are at best difficult to individually quantify. VCS may be a sensitive indicator of toxin-induced neurologic deficits because VCS can be precisely quantified, and because the visual system contains most of the cell types and neurotransmitters found elsewhere in the central nervous system.

The response of PEAS cases to cholestyramine therapy supported the hypothesis that PEAS was a biotoxin-associated illness. Cholestyramine has been used previously for detoxification in case studies and animal models of toxicity, including Kepone [8,21], DDE [57], other organochlorine pesticides [64], polychlorinated biphenyl compounds [11], *Clostridium difficile* toxin [51,56], *E. coli* and *Vibrio cholera* toxins [10,62], a cytotoxin(s) from an unidentified gastrointestinal microorganism(s) [1,40], the mycotoxins, ochratoxin A [22,44], fumonisin B_1 [87], and zearalenone, a *Fusarium* toxin [84], the cyanobacterial toxin, microcystin LR [23], and a toxin from the Chinese herbal product, Jin Bu Huan [18]. The plasma half-life of M1, the active metabolite of Arava™ (leflunomide), a pyrimidine synthesis inhibitor approved by the FDA for treatment of rheumatoid arthritis, was lowered from >1 week to approximately 1 day due to cholestyramine sequestration of M1 from bile in the intestine [3]. This indicated that biliary recycling was a major contributor to the long half-life of M1 [3], as it may be for the toxin(s) which may cause PEAS. Because the only other known therapeutic benefit of cholestyramine treatment is to lower cholesterol levels, and because cholestyramine therapy did not improve VCS in hypercholesterolemia patients, it is likely that the VCS increase and symptom abatement seen in PEAS cases during cholestyramine therapy was due to toxin elimination. However, the process through which cholestyramine therapy was associated with an improvement in health status of the PEAS cases was not demonstrated. Human and animal research is needed to characterize the detoxification process involved in the response to cholestyr-

amine therapy, and to identify the array of toxins for which it is an effective therapy.

Little is known about the modes and mechanisms of action by which VCS deficits and symptoms were induced in PEAS cases. Both the onset and resolution of VCS deficits occurred rapidly in PEAS cases [76]. Rapid alterations in VCS may have resulted from equally rapid changes in blood flow rates in the retina and/or brain. As noted above, the putative *Pfiesteria* toxin, pPfTx, activated or mimicked activation of P2X7 receptors [26,27,45,53]. The activation of P2X7 receptors, as well as leukocyte exposure to the shellfish poison, Maitotoxin, induced release of the proinflammatory cytokine, interleukin-1 beta [86]. Proinflammatory cytokines constricted microvasculature and reduced blood flow rates in rat brain and lung [24,75,77,88]. VCS reductions may have resulted from microvasculature constriction. Blood flow rates in retinal microvasculature were depressed when VCS was reduced in other PEAS cases (Shoemaker—unpublished data). Enhanced rates of toxin elimination due to cholestyramine therapy may have led to a rapid decrease in proinflammatory cytokine levels, increased blood flow rates in microvasculature, and rapid VCS recovery. However, because proinflammatory cytokine levels and blood flow rates were not measured in the PEAS studies discussed above, these possibilities remain speculative.

Other data indicated the need for research on involvement of the proopiomelanocortin pathway of the ventromedial hypothalamus in the illness process. Elevated levels of leptin and depressed levels of alpha melanocyte stimulating hormone (aMSH) were recently observed in a study that demonstrated an association between a PEAS-like illness and exposure to the indoor air of buildings that exhibited water damage and microbial colonization on water damaged surfaces [75]. The “sick building syndrome” (SBS) cases reported multiple system symptoms, showed depressed VCS, and responded to cholestyramine therapy, indicative of a biotoxin-associated illness. The evidence indicated that SBS was induced by inhalation exposure to complex mixtures of fungi, mycotoxins, bacteria, endotoxins, antigens and volatile organic compounds [75]. Leptin has been reported to affect regulation of body weight, hypothalamic activity, and response to feeding [78]. Stimulation of leptin receptors in the ventromedial hypothalamus triggered the release of aMSH. aMSH was reported to exert regulatory effect on pituitary function by controlling the release of growth hormone [5], gonadotrophin [63] and possibly vasopressin [66]. Behavioral functions reported to be influenced by MSH included verbal memory, pain perception [2], attention, and goal-motivated behavior [90]. Additional evidence suggested that MSH had the capacity to affect cerebral protein synthesis, RNA synthesis, and protein phosphorylation, thereby potentially altering the capability of an organism to both evaluate information and interact effectively with its environment [4]. Shoemaker’s unpublished data collected from other PEAS cases indicated that leptin levels were high and that aMSH levels were low during illness and gradually normalized in most cases after cholestyramine therapy. Low aMSH levels in the presence of high leptin levels may have resulted from damage to leptin receptors by toxins or proinflammatory cytokines. This discussion of potential modes of action in PEAS is clearly speculative, and is solely intended to generate research hypotheses.

A final speculation concerned anecdotal reports and observations that some individuals may be more susceptible to chronic PEAS than others. Preliminary data indicated that susceptibility may have been influenced by polymorphisms in genes of the major histocompatibility complex on Chromosome 6 that encoded human leukocyte antigens (HLA). HLA genes were the most polymorphic loci known in humans, with about 100 alleles having been identified [6]. Class II HLA molecules were found only on antigen presenting cells such as macrophages, monocytes, B cells and dendritic cells. The Class II HLA molecules presented exogenous antigenic peptides to CD4 T cells for elimination. If antigen presentation was inappropriate, the T cells would not be sensitized, and the antigen would not be eliminated. Specific HLA alleles were associated with susceptibility to a variety of rheumatic and autoimmune diseases [61]. Preliminary data indicated that specific alleles in the Class II HLA-DR and -DQ loci were over represented in chronic PEAS cases. This suggested that susceptibility to PEAS may be conferred by the inability to naturally eliminate toxins bound to proteins at a sufficient rate. Additional research is needed to identify the causes of differences between individuals in susceptibility to chronic illness following exposure to biotoxins.

Both natural forces, floods and rains, and human activities, inadequate construction techniques and maintenance, contributed to alteration of the indoor environment in the water-damaged buildings associated with SBS [75]. It was less clear, however, how natural forces and human activities may have contributed to *Pfiesteria* episodes. Evidence indicated that *Pfiesteria* blooms were stimulated by inorganic nutrient enrichment [14,16,28], although the effect appeared to be less robust than that associated with blooms of cyanobacteria. Pollutants that alter the balance between populations of alga also influenced *Pfiesteria* activity. A recent report described relationships between *Pfiesteria*, its prey, and predators during exposure to environmentally relevant concentrations of diothiocarbamate pesticides and metals, both alone and in mixtures [85]. The results indicated that some exposures caused lethality among predators of *Pfiesteria* at concentrations below those that affected *Pfiesteria*. The lack of predators led to an increase in the size of the *Pfiesteria* population. Other exposures caused lethality among the prey of *Pfiesteria* at concentrations below those that affected *Pfiesteria*. Elimination of the prey of *Pfiesteria* may force *Pfiesteria* to seek alternative sources of food, such as fish. Pollutant runoff from agricultural and other areas into estuaries may have

effects similar to these observed in the laboratory. Additional research is needed to characterize the forces that are associated with increases and decreases in the frequency of *Pfiesteria* events.

In conclusion, evidence has been presented that supported the hypothesis that PEAS is a biotoxin-associated illness characterized by multiple-system symptoms, a VCS deficit largest at mid-spatial frequencies, cognitive dysfunction, and response to cholestyramine therapy. Research is needed to better characterize the emerging health risk posed by a spatial and temporal increase in the occurrence of toxigenic organisms in the environment. Current or potential threats include *Pfiesteria* in estuaries, cyanobacteria in fresh and brackish water, dinoflagellates in marine water, tick-borne diseases on land, and mixtures of fungi and bacteria in water-damaged buildings. In order to minimize the risk that toxigenic organisms may pose to human health and ecologic viability, it is essential to characterize the natural forces and human activities that are associated with habitat alteration and pollution of the environment (Fig. 1).

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